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TEST METHOD #9

Title: Determination of Soluble Fluoride in Stannous Fluoride -
Calcium Pyrophosphate Toothpaste Supernatants by Fluoride Electrode

Recommended for the Following Systems:

- a. Stannous Fluoride - calcium pyrophosphate
- b. Stannous Fluoride - insoluble sodium metaphosphate

Test Method #9

Determination of Soluble Fluoride in Stannous Fluoride-Calcium Pyrophosphate Toothpaste Supernatants by Fluoride Electrode

Scope

This method is applicable for the determination of soluble, and therefore available, fluoride in stannous fluoride-calcium pyrophosphate toothpastes under normal brushing times and dilutions. The time of slurring and the time until centrifuging begins are critical and must be rigidly adhered to if reproducibility is to be attained. The emf developed by a calibrated Orion fluoride electrode in contact with the toothpaste supernatant is related to the ppm F^- in the supernatant. Stannous fluoride toothpaste supernatants require the addition of an ammonia buffer-EDTA reagent. A calibration curve is also required and is included in this method.

Reagents

| | |
|---|--------------------------------|
| Ammonium Hydroxide | A.C.S. grade |
| Sodium Nitrate | A.C.S. grade |
| Sodium Fluoride | A.C.S. grade |
| Ammonium Chloride | A.C.S. grade |
| (Ethylenedinitrilo) tetraacetic Acid, Disodium Salt ($Na_2EDTA \cdot 2H_2O$) | A.C.S. grade |
| Deionized Distilled Water | Laboratory supply, oxygen-free |

Apparatus

| | |
|---------------------------------|---|
| Pipettes | 5, 25, 50 ml. |
| Magnetic Stirrer | Labline "Magne-stir" or equivalent |
| Stirring Bars | Teflon coated, 7/8 inch, or equivalent. |
| Polyethylene Bottles | 250, 500 ml. |
| Volumetric Flasks | 50, 250 ml. |
| Pasteur Pipette | |
| Expanded Scale pH meter | Beckman Expandomatic or equivalent. |
| Fluoride Specific Ion Electrode | Orion Research, Inc., 11 Blackstone St., Cambridge, MA 02139; Model 94-09-00 (Available from Fisher Scientific Products, etc.) |

OR

Combination Fluoride & Reference Electrode Model No. 96-09 (Orion)

NOTE: Two electrodes should be on hand if heavy use is anticipated.

| | |
|--|--|
| Balance | Harvard Trip or equivalent |
| Single Junction Reference Electrode | Orion Research, Inc., Model No. 90-01 |
| Electrode Holder | Orion Research, Inc., Model No. 92-00-01 |

| | |
|------------------------|--|
| Centrifuge, high speed | International Model CS, UV, 2N, or 2-EXD with high-speed attachment. |
| Centrifuge Tubes | 25 ml, International No. 298 |
| Plastic Beakers | 100 ml disposable |
| pH paper | Hydrion, or equivalent, with 0.2 pH interval or less. |
| Stirring Rods | 8-inch, with about 2-inch piece of Tygon tubing slipped over one end, such that an inch of Tygon extends beyond the glass. |
| Graph paper | Codex Brand No. 31,286 (two-cycle semi-log graph paper) |

Preparation of Oxygen-free distilled water

Distilled water is placed in an Erlenmeyer flask or gallon jug and a nitrogen line, ending in a gas dispersing tube, is inserted into the water. Vigorously bubbling nitrogen through the water for a minimum of 30 minutes will render the water sufficiently oxygen-free for this analysis.

Sodium Fluoride Stock Solution (0.500M)

Dry about 10 g sodium fluoride at 105°C for 3 hours. Store the sodium fluoride in a desiccator and allow to cool. Weigh out 5.249 g of the dried reagent, dissolve in distilled deionized water, dilute to 250 ml in a volumetric flask. Minimize the time of contact with glass. Store in a 250 ml polyethylene bottle.

Sodium Nitrate Stock Solution (1.00M)

Weigh out 21.25 g of sodium nitrate, dissolve in distilled deionized water, dilute to 250 ml in a volumetric flask.

Preparation of Sodium Fluoride-Sodium Nitrate Calibration Solution (0.005 M NaF, 0.1 M NaNO₃)

Pipette 5 ml of the sodium fluoride stock solution and 50 ml of the sodium nitrate stock solution into a 500 ml volumetric flask. Dilute to the volume with distilled deionized water. Store in polyethylene bottle.

Stock Ammonia Buffer Solution

Place 67.5 g ammonium chloride in a one-liter flask and dissolve with about 250 ml of distilled deionized water. Add 570 ml of concentrated ammonium hydroxide to the flask and dilute to volume with distilled deionized water. Store in a polyethylene bottle. Discard solution within seven days after preparation. Use of solution older than seven days may result in erroneous readings.

Preparation of Dilute Ammonia Buffer-EDTA Reagent Solution (0.1 M EDTA)

Place 37.2 g of (ethylenedinitrilo) tetraacetic acid disodium salt (EDTA) in a one-liter volumetric flask. Add 250 ml of the stock ammonia buffer to the flask and swirl to dissolve the EDTA. Dilute to volume with distilled deionized water. Store in a polyethylene bottle.

Potential Scale of pH Meter

The Beckman Expandomatic pH meter should be used in the expanded scale and -MV modes. The potential range necessary for fluoride activity measurements is 0 to -200 MV. A standard solution of 0.005M NaF, 0.1M NaNO₃ will be arbitrarily assigned a reading of -100 MV; that is, the meter should be adjusted to read -100 MV (the center of the MV scale) when the fluoride and reference are contacting the calibrating solution. Before the electrodes are removed from a solution the "STANDBY" button on the meter is pressed.

Calibration of Orion Fluoride Specific Ion Electrode

The instruction book for the electrode should be read in order that the analyst may become familiar with proper electrode handling and optimum operating conditions. The fluoride electrode must be calibrated every two weeks to establish the potential response versus concentration of free fluoride. The Orion single junction reference electrode should be used as the reference electrode. The following three solutions are necessary and sufficient for calibrating the electrode:

1. 0.05 M NaF, 0.1 M NaNO₃
2. 0.005 M NaF, 0.1 M NaNO₃
3. 0.0005 M NaF, 0.1 M NaNO₃

They can be prepared from stock solutions. Solution 2 (0.005 M NaF, 0.1 M NaNO₃) should be read first and the potential adjusted to read - 100.0 MV (estimated to the nearest 0.5 MV). The solutions should then be read in the following order 2, 1, 2, 3, 2; the potential of 2 should not drift more than ± 1 MV and should be readjusted to -100.0 MV in any case.

The solutions should be stirred but not so vigorously as to allow bubbles to form and contact the fluoride electrode membrane crystal. At least one minute should be allowed for the electrode to reach a steady reading. A plot of MV versus log concentration on semi-log paper should yield a slope of 59 ± 1 MV. (That is, the potential difference between solutions 1 and 2, and 2 and 3 should be 59 ± 1 MV). If the electrode does not behave in this manner, it should be exchanged for a new electrode under the warranty terms with Orion Research, Inc.

Preparation of Supernatants

Two samples can conveniently be prepared at the same time. Discard the first inch of toothpaste from the tube, then weigh 10.0 grams of each paste into 100 ml beakers. Pipette 30 ml of oxygen-free distilled water into each of the beakers and then set a clock-timer for 5 minutes before starting the slurring. Start the timer and then, using a separate Tygon-tipped stirring rod in each beaker, alternately slurry each sample (10-15 seconds each) until 2 minutes have elapsed. Pour 22-25 ml of the uniform slurries into centrifuge cups, taking care to balance the two cups very precisely. Five minutes after starting to slurry the pastes (by the clock-timer), start the centrifuge and spin the slurries for 30 minutes at 11,000 r.p.m. Two other pastes may be started at this time, and the centrifuge may be stopped to add these two samples at the end of their 5-minute time period. All four samples are then centrifuged for 30 minutes.

Operation

Pipette 15 ml of the toothpaste supernatant into a 100 ml plastic beaker. Pipette 10 ml of the dilute ammonia buffer-EDTA reagent solution into the same beaker. Before measuring the fluoride activity of the supernatant, the electrode should be calibrated to read -100 MV when measuring the fluoride activity of the

calibrating solution, 0.005 M NaF, 0.1 M NaNO₃. The fluoride and reference electrodes are then lowered into the test solution and the -MV button pressed. Take the potential reading after one minute of stirring to allow the electrode to reach a steady value. The potential should be read to the nearest 0.5 MV and that value recorded. The electrodes should then be thoroughly washed with distilled water, especially the membrane crystal of the fluoride electrode. The electrodes are then gently wiped dry with absorbent tissue. The calibrating solution is then remeasured. If the potential is more than ± 1 MV different from -100 MV, it should be adjusted to -100 MV and the potential of the supernatant remeasured.

Determination of Fluoride Concentration in Supernatants as ppm

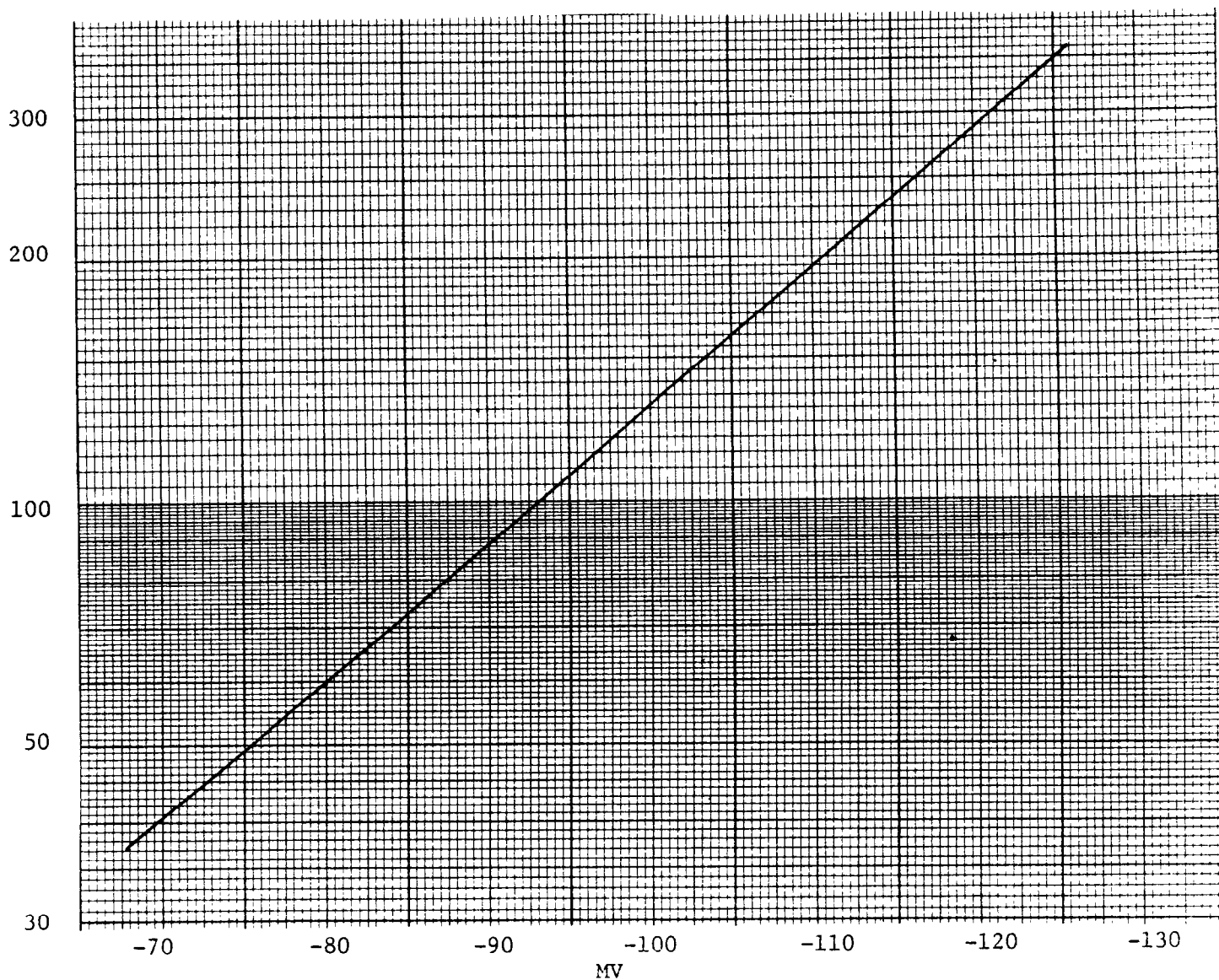
A calibration curve similar to the one shown in this method should be used. The "ppm F" is the vertical or log-scale and "MV" is the horizontal axis. When the calibrating solution is adjusted to read -100 MV, the measured potential of the test solution is found on the curve, and the ppm F⁻ in the original supernatant is read and recorded. This calibration curve has a dilution factor of 15/25 incorporated into it, so no correction is necessary. The calibration curve may be prepared by plotting the following data on two-cycle semi-log graph paper and drawing a straight line through the points.

| <u>Log Scale</u> <u>ppm F⁻</u> | <u>Linear Scale</u> <u>MV</u> |
|--|----------------------------------|
| 60 | -80.0 |
| 130 | -99.7 |
| 280 | -119.5 |

Temperature Control of the Test Solutions

In order to prevent the test solutions from being heated by the magnetic stirrer, insulate the test solution. Two suggested methods are (1) place a section of cardboard on the stirrer or (2) use a triple cup thickness. The potential of the calibrating solution and each test solution should be measured at room temperature (70-80°F).

Calibration Curve for Soluble Fluoride
in Stannous Fluoride - Calcium Pyrophosphate Toothpaste



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STANDARDS FOR FLUORIDE DENTIFRICES
TEST METHOD #10
DETERMINATION OF SOLUBLE FLUORIDE (IONIC)

Recommended for the following systems:

- a. Stannous fluoride and silica abrasive.

TEST METHOD #10

DETERMINATION OF SOLUBLE FLUORIDE (IONIC)

Principle

The sample is a centrifuged aqueous extract of toothpaste.

The fluoride content of this solution is determined by the potentiometric technique of multiple standard addition. In this technique incremental volumes of a standard fluoride solution are added to the sample solution. The potentials developed at a fluoride sensitive electrode vs. a suitable reference electrode are observed at each step. These stepwise changes in potential, measured in millivolts, are related to the original fluoride concentration of the sample solution by a computation derived from Nernst's equation (*).

$$\text{Equation (1)} \quad C_x = \frac{C_s}{10^{\frac{\Delta E}{\text{slope}}} \left[1 + \frac{V_x}{V_s} \right] - \frac{V_x}{V_s}}$$

Term C_x is the unknown concentration, C_s is the fluoride concentration in the standard solution, ΔE is the potential change caused by the addition of the known increment of fluoride solution, "slope" is the slope of the Nernst equation, V_s is the volume of fluoride standard added, and V_x is the original volume of sample solution.

Apparatus

Centrifuge, Servall Type SP: Ivan Sorvall, Inc., Norwalk, Conn.
Centrifuge tubes, round bottom, polypropylene, 50-ml, such as SGA Scientific Inc. Item No. C-3512-2.

Centrifuge tube closures to fit 50-ml centrifuge tubes, such as SGA Scientific Inc. Item No. C-3512-4.

Corning Digital 112 Research pH/mV Meter.

Orion Fluoride Specific-Ion-Sensitive Electrode 94-09A.

Orion Single-Junction Reference Electrode 90-01 and a supply of Orion Reference Electrode Filling Solution 90-00-01. DO NOT USE ANY OTHER FILLING SOLUTION!

Magnetic Stirring Apparatus.

Erlenmeyer flask, 50-ml.

Burets, 10-, 25- and 50-ml, meeting Class A specifications.

Graduated cylinders, 50-ml.

Volumetric Flasks, 250-ml and 1000-ml sizes.

Pipets, 10-ml, 20-ml, 25-ml sizes, Class A.

Heath EUA-20-12 pH/mV Test Box.

* Karlberg, B. Anal. Chem. 43, 1911-1913 (1971).

Reagents

Sodium Hydroxide Pellets, ACS Reagent Grade. Prepare a liter of Sodium Hydroxide Solution, 10% w/v in distilled water. Store in a polyethylene bottle.

Glacial Acetic Acid, ACS Reagent Grade. Prepare 2 liters of Acetic Acid Solution, 10% v/v in distilled water.

Phenolphthalein Indicator Solution, prepare as in A.M. 0.020 for laboratory use.

Sodium Fluoride, ACS Reagent Grade, preferably Fisher Certified S-299. Prepare the solution shown below.

Master Fluoride Solution (2500 ppm w/v Fluoride, pH 5).

Dissolve 5.526 grams of reagent grade ACS-specification sodium fluoride in water, add 40 ml of 10% w/v sodium hydroxide solution, then add 80 ml of 10% v/v acetic acid solution and mix. Transfer the mixture quantitatively to a 1000-ml volumetric flask. Make the contents of the flask to the mark with water near 25°C and mix thoroughly.

Transfer the solution to a polyethylene bottle for storage.

Procedure

A few instrumental preliminaries are needed. The fluoride electrode is preferably stored dry, covered with a cap to protect the fragile crystal membrane. The reference electrode is also stored with a protective cap to prevent evaporation. Remove these caps, inspect both electrodes for obvious mechanical damage, and if none is evident immerse the pair in stirred distilled water. Refill the reference electrode if necessary, using only the Orion filling solution.

The Corning meter may be left on permanently. If there is any suspicion that the meter is not functioning properly, use the Heath test box to check the meter, remembering that the Corning meter is more accurate than the test source, and this procedure will only detect gross error.

Preheat a hot plate while samples are being extracted. An 8" Chromalox 1500-watt hot plate is recommended for fastest warmup time.

First, prepare the "available" fluoride extract. Weigh 3.5 grams of toothpaste, to an accuracy of 1 mg, into a centrifuge tube.

Keep the centrifuge tube capped as much as possible during the entire extraction procedure.

Add from the 50 ml buret 1- to 2-ml increments of oxygen-free water, thoroughly mixing with the paste (use a glass rod) after each addition until a smooth slurry is obtained (about 10 ml of water). Continue the addition of water until the total volume added is 10 times the weight of paste taken.

Mix by stirring, rinse off the glass rod with 1 or 2 ml of oxygen-free water from the buret, cap the tube, and centrifuge at 5000 rpm for 15 minutes. Read the total volume of water added from the buret to the nearest 0.1 ml.

Immediately decant the supernatant liquid into a 50-ml Erlenmeyer flask. Aliquots of this solution are used for "available" fluoride determinations.

The fluoride in the extract is then determined.

Tare a 300-ml Pyrex beaker. Accurately weigh into it by difference 20-25 grams of "available fluoride" extract. Add 10 ml of 10% w/v sodium hydroxide solution from a pipet. Add 50 ml of water from a graduate.

The next operation is slightly hazardous, and appropriate eye protection is demanded. Beaker tongs and gloves must also be used. Using a 10-inch glass stirring rod for constant stirring to avoid violent bumping, heat the contents of the beaker to a rolling boil for approximately a one-minute boiling period. Vigorous foaming will occur, and the mixture will become almost clear. Without interrupting the stirring, lift the beaker from the hot plate until the foaming subsides. Stop stirring when foaming stops, then let the mixture cool for approximately ten minutes.

Quantitatively transfer the mixture from the beaker to a 250-ml volumetric flask without using excessively large amounts of water. A small quantity (several drops) of phenolphthalein indicator solution may be added to the flask if desired. Pipet 20 ml of 10% v/v acetic acid solution into the flask, swirling it gently as the addition is made. Neutralization will occur before the entire quantity of acetic acid solution has been added. Make the contents of the flask to the mark with water and mix. Invert the beaker on a towel for a few minutes to drain.

Without using any additional water, transfer the contents of the flask back into the original beaker, draining the flask well by use of a small ring clamp. A small transfer error is involved here, but its size is trivial. Clean the volumetric flask before the film of solution on its surface has dried, or it will be difficult to clean. Cover the beaker with a watch glass or Parafilm until the determination can be completed. Slow precipitation of hydrous silica may occur. Wait at least $\frac{1}{2}$ hour to allow nucleation of hydrous silica to begin. The next operation may be performed immediately or within 24 hours irrespective of the sample appearance. Overnight standing ordinarily results in copious precipitation of hydrous silica, but this has no noticeable effect on the results.

The multiple additions procedure requires several precautions. The sample temperature must be within a few degrees of room temperature, and most stirrers require thermal insulation to keep the sample from heating. A square of plastic foam covering the stirrer top is advised. The electrode immersion depth should be fixed, and the stirring rate should be fixed but not vigorous enough to create a vortex. The electrodes should be wiped gently with a tissue before every immersion. If a blank is to be analyzed because of suspected fluoride contamination, the blank must be the first analysis of the day, because the electrode has a definite "memory". Analysis of blanks is not necessary except when reagent lots are changed.

Record the mV reading of the electrode pair in distilled water. Immerse the electrodes in the stirred sample solution, and follow the timing schedule below. Precise timing is not required, but these minimum times must be observed.

- (1) Record the initial potential after 5 minutes.
(Exception: Wait ten minutes if this is the first sample of the day).
- (2) Add 1.00 ml of 2500 ppm fluoride master solution from a buret, and record the potential 2 minutes after the addition is complete.
- (3) Four additional 1.00-ml volumes of 2500 ppm fluoride master solution are added next, so for buret readings of 2.00, 3.00, 4.00, and 5.00 record the potential 1 minute after the addition is complete.
- (4) After each sample, rinse the electrodes briefly with distilled water, then return them to stirred distilled water until the reading is approximately 120 mV higher than the initial reading from a fluoride-containing toothpaste. Record these readings also. A properly functioning fluoride electrode should attain these responses in 2 minutes or less. It is not necessary or desirable to wash the electrode exhaustively with water between analyses, because of the "memory" effect.

Computations

Fluoride is calculated in accordance with Equation (1), above. Five values of this equation are computed for each of five additions of a standard fluoride solution. The mean of these and its estimated standard deviation are calculated, too.

C_x is the unknown fluoride concentration of the sample solution.

C_s is the fluoride concentration of the standard solution.

ΔE_1 is the incremental potential change in millivolts observed upon addition of the first volume of standard fluoride.

V_x is the original volume of the sample solution in milliliters.

V_s is the totally added volume of the standard fluoride solution in milliliters.

A value of -59.16 millivolts per decade change of fluoride activity may be assumed. It is convenient to express fluoride concentrations in micrograms F per milliliter. Results are then recomputed to the paste basis.

(The equation is not as formidable as it appears to be. If a fluoride electrode has a negative logarithmic response to fluoride activity, it is intuitively obvious that fluoride activity will be an inverse antilogarithmic function of electrode potential. The remaining terms in the equation are volume corrections).

A volume-corrected Gran Function (2) is also calculated for each of the five additions. This antilogarithmic function provides additional confidence in the performance of the method.

The true slope and standard potential of the electrode system can be approximated from a calibration line by nonlinear regression analysis of calibration data of the form $y = a + b \log_{10} x$. However, these are best determined by the calculation of Brand and Rechnitz (3), which also provides an independent estimate of fluoride concentration. This computation is a formidable numerical analysis routine demanding the facilities of a computer. The earlier computations are readily program-mable.

Example

Assume a 2.500 gram sample of dentifrice containing its target level of 1000 μg F per gram of paste, or a total of 2500 μg F. This 2500 μg quantity of F dissolved in 250 ml total volume gives a fluoride concentration of 10.000 micrograms per milliliter. Now add five separate 1.00-ml increments of a standard fluoride solution with 2500 μg F/ml. Observe the response of a fluoride electrode with perfect slope (-59.16mV) and a standard potential of -180 mV at 1 molar F activity. The following conditions should prevail:

| <u>ml added</u> | <u>μg F present</u> | <u>Volume, ml</u> | <u>ppm F</u> | <u>pF Molarity</u> | <u>E in Millivolts</u> |
|-----------------|---|-------------------|--------------|--------------------|------------------------|
| None | 2500 | 250 | 10.00 | 3.27875 | 13.9711 |
| 1.00 | 5000 | 251 | 19.9203 | 2.97946 | -3.7353 |
| 2.00 | 7500 | 252 | 29.7619 | 2.80509 | -14.0507 |
| 3.00 | 10,000 | 253 | 39.5257 | 2.68187 | -21.3403 |
| 4.00 | 12,500 | 254 | 49.2126 | 2.58668 | -26.9722 |
| 5.00 | 15,000 | 255 | 58.8235 | 2.5092 | -31.5556 |

| <u>ml added</u> | <u>Delta EqmV</u> | <u>Volume-Corrected Gran Function</u> | <u>Computed F $\mu\text{g}/\text{ml}$ of Original Solution by Equation (1)</u> |
|-----------------|-------------------|---|---|
| None | (Initial) | - | - |
| 1.00 | -17.71 | 2.0003 | 9.9972 |
| 2.00 | -28.02 | 2.9998 | 10.001 |
| 3.00 | -35.31 | 3.9998 | 10.0007 |
| 4.00 | -40.94 | 4.9994 | 10.0016 |
| 5.00 | -45.53 | 6.0008 | 9.9984 |

The last column obviously confirms the target level of 1000 μg F per gram on the original sample of dentifrice in this example. Available fluoride calculations are similar.

- (1) Karlberg, B. Anal. Chem. **43** 1911-1913 (1971).
- (2) Gran, G. Analyst **77** 661-671 (1952)
- (3) Brand, M.J.D., and Rechnitz, G.A. Anal. Chem. **42** 1172-1177 (1970).

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #11

TITLE: DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

Recommended for the following systems:

- a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate
- b. Sodium Monofluorophosphate/Dicalcium Phosphate
- c. Sodium Monofluorophosphate/Alumina
- d. Sodium Monofluorophosphate/Silica
- e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #11

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

PRINCIPLE

The method for total soluble fluoride is based on the same principle as for total fluoride, except that an aqueous extract is used instead of an acid extract.

APPARATUS REQUIRED

Same as for the total fluoride method.

REAGENTS REQUIRED

Same as for the total fluoride method except for the absence of perchloric acid.

PROCEDURE

1. Accurately weigh (to the nearest milligram) 20 grams of sample into a 100 or 150 ml beaker.
2. Add 60 ml of water. Slurry to disperse the sample and break up any lumps.
3. Quantitatively transfer to a 100 ml volumetric flask and dilute to volume with water.
4. Mix vigorously using a magnetic stirring bar and stirrer for at least 30 minutes.
5. Centrifuge a portion of the above slurry until clear.
6. Pipette 15.0 ml of the clear supernatant liquid into a 500 ml volumetric flask and dilute to volume with water. Mix well.
7. Transfer a portion to the plastic sample cups. See Note A.
8. With the fluoride manifold on the proportioning pump and all the lines in water lower the roller head to start the instrument. Start the vacuum pump and adjust to 5 or 6 inches of Mercury. Keep the vacuum constant once the test is started.

9. Run water through the lines for a few minutes to check the flow. Dip all lines in their respective solutions.
10. Set the sampler table with the 20, 1-2 samples per hour cam and load the tray with 3 - 3.5 ml plastic cups.
11. The cups are filled alternately with standards and samples. Since each laboratory's needs will vary, no fixed procedure for this step can be made. It is recommended that the first 22 cups, which will approximate a one hour run, be filled as follows:
See Note B.

| | | |
|--------------|------|------------------------|
| Cups 1 - 4 | 1.12 | Standard (mg F/200 ml) |
| Cups 5 - 10 | | Sample Solutions* |
| Cups 11 - 13 | 1.44 | Standard (mg F/200 ml) |
| Cups 14 - 19 | | Sample Solutions* |
| Cups 20 - 22 | 1.28 | Standard (mg F/200 ml) |

* Two cups are filled for each sample and the average of the two analyses used in the calculation. Single analyses may be run once the instrument has been shown to yield consistent results.

12. The succeeding cups are filled alternately with samples (in duplicate), three 1.44 standards, samples (in duplicate), and three 1.12 standards.
13. Turn on the colorimeter and recorder.
14. When the baseline is established, set the absorbance at 0.01 and start sampling, making certain that the sampling probe dips well into the cups. It is essential that a steady bubble pattern of small bubbles be established after the working reagent is added. A steady baseline will be assurance of this.
15. After the last sample has been aspirated, turn off the sampler and discard the cups.
16. After the last sample trace appears on the recorder chart, dip all the lines in water and flush the system.
17. After the instrument lines are washed for about 15 minutes, lift up the roller head, release the manifold and stop the vacuum pump.
18. Remove the recorder chart and record the absorbance values of each peak at the peak height. Ignore the first peak from the 1.12 standard.

CALCULATIONS

The calculations are performed on sets representing each hour of running time (Steps 11 and 12).

1. Calculate the average absorbance of each standard by adding the absorbance values and dividing by three. The first cup peak must be ignored.
2. For each standard calculate the K value as follows:

$$K = \frac{\text{Milligrams F per 200 ml}}{\text{Average Absorbance of each Standard}}$$

3. Calculate the average K_{AV} value for each hour run by adding the standard K values and dividing by the number of standards represented. It is important to determine the K_{AV} for each hour of operation and to use the value obtained for calculation of results for samples included in that run. See Note C.
4. Calculate the Total Fluoride as follows:

$$\frac{(K_{AV} \times A) \times 100 \times 0.06}{S} = \text{Total Soluble Fluoride as F}$$

Where K_{AV} = Average K value (calculation 3)

A = Average absorption of samples

S = Sample weight in milligrams (Step 1).

NOTES:

- A. The placebo fluoride standards used for soluble fluoride are the same as those used in the total fluoride method. If the soluble fluoride is less than 1 mg/200 ml, then appropriate, lower standards should be used.
- B. The indicated order in which the cups are filled is only given as an example and the samples and standards may be interchanged as desired.
- C. It has been observed that the system may need to be standardized continuously. Thus, hourly averages of K for standardization are suggested. For instance, a run of 3 hours would require three average K values and each K value would be used in calculating the % fluoride content of the inclusive unknowns.

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STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #12

TITLE: DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

Recommended for the following systems:

- a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate
- b. Sodium Monofluorophosphate/Dicalcium Phosphate
- c. Sodium Monofluorophosphate/Alumina
- d. Sodium Monofluorophosphate/Silica
- e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #12

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

PRINCIPLE

Total Soluble Fluoride is based upon the removal of water insoluble ingredients by centrifugation. The supernatant liquid is in the presence of calcium oxide. The fluoride is distilled from a perchloric acid solution of the ashed residue and reacted in solution with Eriochrome Cyanine R and Zirconyl Chloride. The bleaching effect of the fluoride on the Zirconium lake is measured spectrophotometrically and the fluoride level is obtained by reference to a standard graph.

METHOD

APPARATUS REQUIRED

1. Steam Distillation Apparatus of borosilicate glass with band heater as shown in Figure 1, LaPine Scientific Co., Irvington-On-Hudson, N. Y.
2. Glas-Col Heating Mantle, hemispherical to fit 2000 ml flask.
3. Variable Transformers (2) such as the "Powerstat" Type 116, Superior Electric Co., Bristol, Conn., or "Variac", Type V-5, General Radio Co., Cambridge, Mass. These transformers are available from most laboratory supply houses.
4. Centrifuge, capable of speeds up to 10,000 rpm.
5. Centrifuge tubes, Nickel, 1" x 4", (2).
6. Mechanical stirrer.
7. Nickel dish - 30 ml.
8. Muffle furnace.

REAGENTS REQUIRED

Zirconyl Chloride Solution (ZrOCl_2)

Dissolve 0.265 gram of Zirconyl Chloride Octahydrate ($\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$) in 50 ml of water. Add 700 ml of conc. Hydrochloric Acid and cautiously dilute to volume with water at room temperature in a 1000 ml volumetric flask. Mix well.

NOTE:

0.220 gram of Zirconyl Nitrate Dihydrate ($\text{ZrO}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$) may be used in place of Zirconyl Chloride Octahydrate.

Silver Perchlorate Solution (AgClO_4) 50%

Dissolve 250 grams of anhydrous Silver Perchlorate (AgClO_4) in 400 ml of water and dilute to 500 ml.

Eriochrome Cyanine R Solution

Dissolve 1.8 grams of Eriochrome Cyanine R (obtainable from Geigy Dyestuffs Div., Geigy Chemical Corp., N. Y., N. Y.) in 100 ml of water and dilute to 1000 ml.

Sodium Hydroxide Solution (NaOH), 5%

Dissolve 5 grams of Sodium Hydroxide in 50 ml of water and dilute to 100 ml.

Calcium Oxide (CaO): The grade obtainable from Fisher Scientific Co., Catalog No. C-117 (0.005% Fluoride) is recommended.

Sodium Fluoride (NaF) Reagent Grade.

Perchloric Acid (HClO_4), 70%.

Hydrochloric Acid Solution (70 ml conc. HCl + 30 ml water) -
Baker or Mallinckrodt HCl is recommended.

PREPARATION OF STANDARD CURVE

1. Accurately weigh 0.8842 gram of Sodium Fluoride and transfer to a 1000 ml volumetric flask. Add 500 ml of water and shake to dissolve the sample. Dilute to volume with water at room temperature and mix well. Store in a polyethylene bottle.
2. Pipet 10 ml of the sample solution from step 1 into a 1000 ml volumetric flask. Dilute to volume with water at room temperature and mix well. Each ml of this solution contains 0.00400 milligram (4 micrograms) of F. Store in a polyethylene bottle.
3. Using a buret, deliver 5.00 ml, 7.50 ml, 10.00 ml, 12.50 ml and 15.00 ml aliquots of the dilute solution from step 2 into separate 100 ml volumetric flasks. Add approximately 50 ml of water. Pipet 5 ml of Eriochrome Cyanine R solution and 5 ml of Zirconyl Chloride solution into each flask. Dilute to volume with water and mix well.
4. Using 1 cm cells in the Beckman DU Spectrophotometer, measure the absorbance of each solution from step 3 at 527.5 millimicrons. Use the reference solution as a blank. See step 14.

PROCEDURE

1. Accurately weigh 20 ± 0.2 grams (to the nearest 0.1 milligram) of sample into a 150 ml beaker. Add 60 ml of water and mix thoroughly with a stirring rod and then with a magnetic stirrer for 15 minutes.
2. Quantitatively transfer the dispersion to a 100 ml glass stoppered mixing cylinder using water to complete the transfer. Dilute to the 100 ml mark with water, stopper the cylinder and shake vigorously.
3. Transfer the dispersion from step 2 in equal amounts to 2 Nickel centrifuge tubes and centrifuge at 10,000 rpm for 20 minutes.
4. Pipet 10 ml of the clear supernatant liquid into a 30 ml Nickel dish. Add 0.5 grams of CaO and mix well.
5. Place the dish on a steam bath first to dry, then under a heating lamp and evaporate to dryness and char. Finally ash in a muffle furnace maintained at $560-600^{\circ}\text{C}$ for 1/2 hour. At no time should the sample be permitted to ignite and burn with a flame.
6. Cool to room temperature and transfer the main contents of the dish with about 30 ml of water to the fluoride distilling flask containing approx. 0.5 gm of glass wool.
7. Add about 10 ml of 70% Perchloric acid to the dish. Mix, and with the aid of water, transfer to the distilling flask. Add an additional 40 ml of the 70% Perchloric acid and 1 ml of Silver Perchlorate to the sample solution in the flask. Place a 500 ml volumetric flask under the distillate outlet.
8. Turn on the transformer connected to the heating mantle surrounding the steam generator and set at the highest dial reading (about 130). Leave the Hoffman clamp open until the water boils.
9. Turn on the transformer connected to the band heater and set the dial at 100.
10. When the temperature in the distilling flask is approximately 130°C and the water is boiling in the steam generator, steam is admitted to the distilling flask by closing the Hoffman clamp. The temperature in the distilling flask is maintained at $130^{\circ} \pm 2^{\circ}\text{C}$ by temporarily turning off the transformer connected to the band heater if the temperature reaches 137°C and turning it on again if the temperature drops to 135°C . The installation of an automatic temperature control, known as the "Therm-O-Watch," to the band heater and thermometer of the distillation unit will allow the operator to perform other duties while the distillation is in progress. The "Therm-O-Watch" unit (Model S-6) is obtainable from Instruments for Research and Industry, 108 Franklin Avenue, Cheltenham, Pa.

11. Collect about 450 ml of distillate. Turn the unit off by opening the Hoffman clamp and shutting off both transformers.
12. Neutralize the distillate to litmus by the dropwise addition of 5% Sodium Hydroxide solution and dilute to volume with water at room temperature. Mix well.
13. Pipet a 10 ml aliquot of the solution from step 12 into a 100 ml volumetric flask. Add approximately 50 ml of water. Using micro burets, measure 5 ml of Eriochrome Cyanine R solution and 5 ml of Zirconyl Chloride solution into the flask. Dilute to volume and mix well.
14. Using 1 cm cells in the Beckman DU Spectrophotometer, measure the absorbance of the solution at 527.5 millimicrons versus a reference solution prepared as described in step 15.
15. The reference solution is prepared as follows: Measure 3.00 ml of Eriochrome Cyanine R solution into a 100 ml volumetric flask. Dilute to about 85 ml with water and add from a pipet 10 ml of 70/30 HCl solution. Dilute to volume and mix well. In some cases variations in reagents may result in an abnormal standard curve (Low Absorbance readings for each standard). In this case dilute the reference solution with a known and accurately measured volume of water so that the absorbance readings are on scale. The absorbance of the 5 ml standard should be between 0.4 and 0.5 . The same reference solution used to prepare the standard curve must be used in analyzing samples.
16. From the absorbance, determine the milligrams of Fluoride by reference to the Standard Curve.

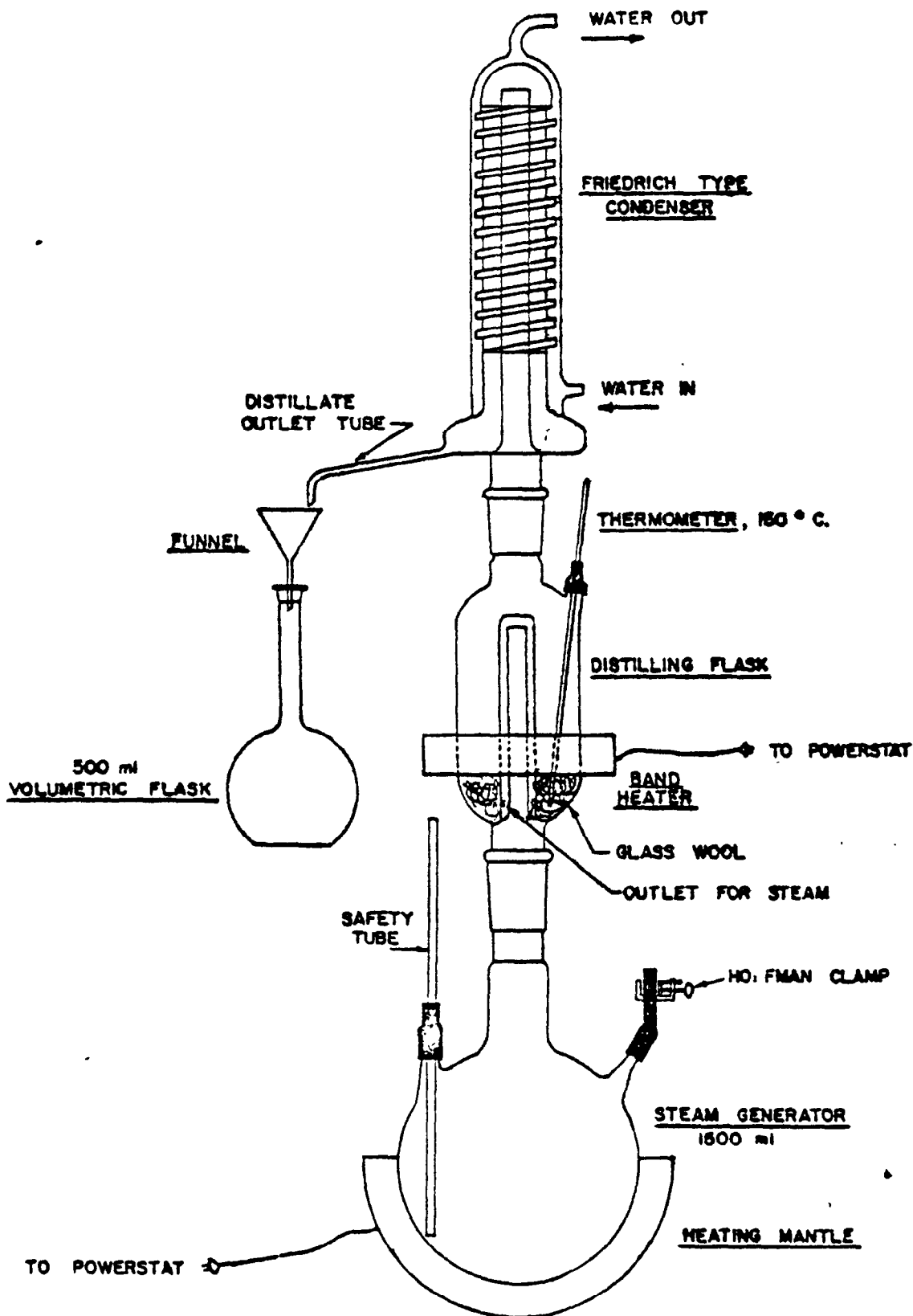
CALCULATION

1.
$$\frac{(\text{mg F from Curve}) \times 100}{\text{Wt. of Sample} \times 1000} = \% \text{ Total Soluble Fluoride as F}$$

in Aliquot in grams

step B.

FIGURE I
FLUORIDE STEAM DISTILLATION APPARATUS



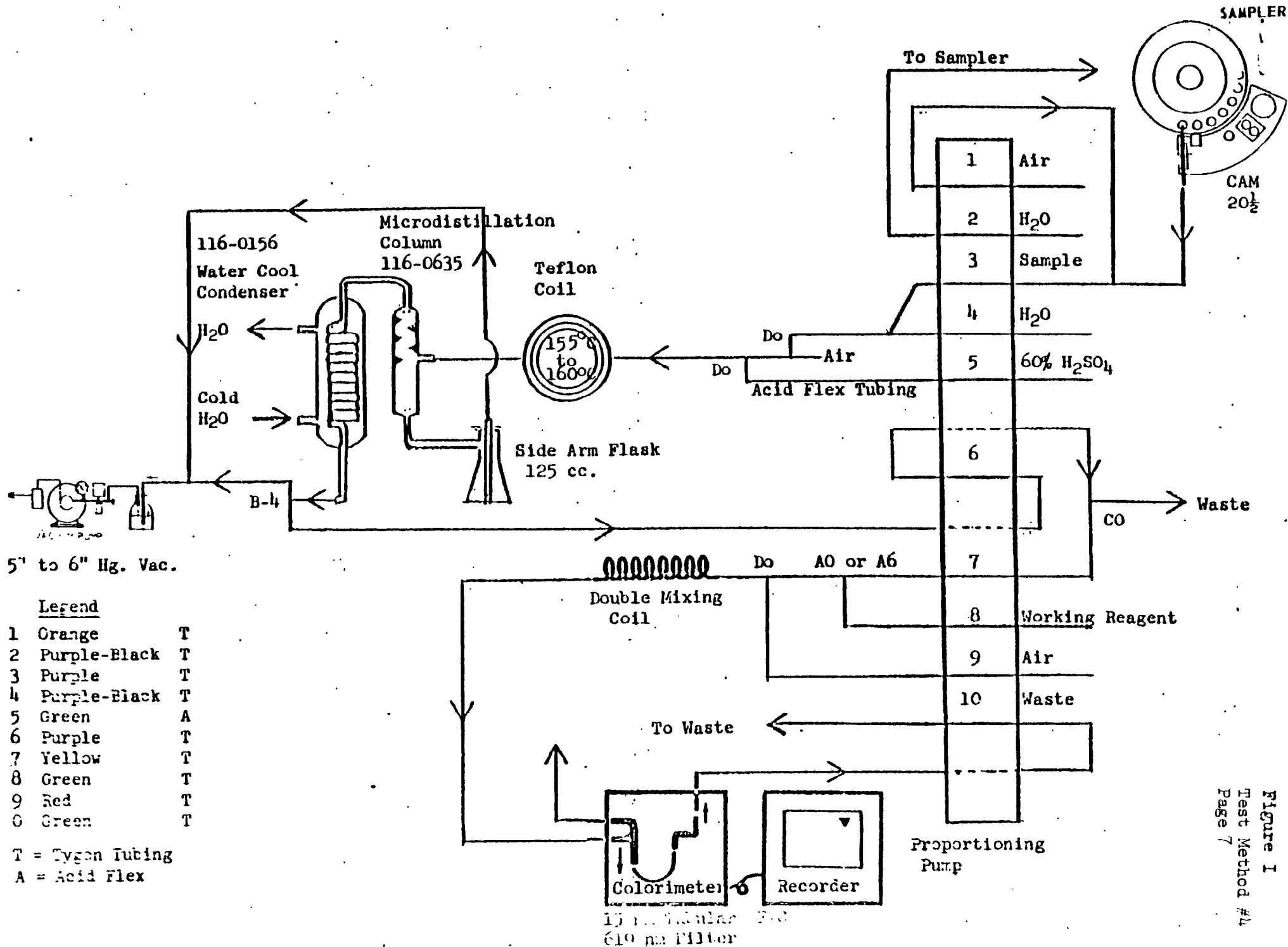


Figure 1
Test Method #4
Page 7

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STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #13

TITLE: DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

Recommended for the following systems:

- a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate
- b. Sodium Monofluorophosphate/Dicalcium Phosphate
- c. Sodium Monofluorophosphate/Alumina
- d. Sodium Monofluorophosphate/Silica
- e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #13

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

In addition to the previous methods, Total Soluble Available Fluorine can be calculated by adding the results obtained for soluble PO_3F^- ion and soluble F^- ion.

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STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #14

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE
IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #14DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE
IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICEObjective:

The objective of this method is to measure the total soluble available fluorine in a dentifrice.

ReagentsSuggested Type or Source

| | |
|---------------------------|------------------------|
| n-Pentane 99% | Tridom Chemical |
| Toluene 99% | MCB Chromatoquality |
| HCl Conc. | Baker Analyzed reagent |
| Trimethylchlorosilane 99% | Tridom Chemical |
| NaF | Baker Analyzed reagent |
| Test tubes (screw-cap) | S.G.A. |
| Pipettes | S.G.A. |

ApparatusSuggested Type or Source

Gas Chromatograph Model 5710A Hewlett-Packard
with dual flame ionization
detector

Column: 6 ft. 1/8" O.D. stainless steel packed with
25% DC-200 on Chromosorb NAW 80 - 100 mesh.

Temperature: Column = 90°C,
Detector = 250°C
Injection Port = 150°C

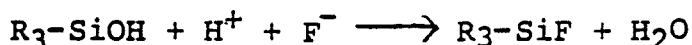
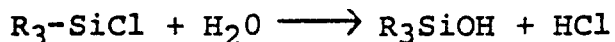
| | | |
|----------|----------------------|------------------|
| Carrier: | Nitrogen = 30 ml/min | Matheson Gas Co. |
| | Hydrogen = 14 psig | Matheson Gas Co. |
| | Air = 24 psig | |

Range: 64 x 10

Sample size: 0.6 ul of top layer

Notes on Method

This method is essentially the same as published by Cropper and Puttnams (J.S.C.C. 21,533,1970) for the determination of total fluoride in dental creams. It is based on an original publication of Bock and Semmler (Anal.Chem. 230,161,1967) and involves the following reaction:



The alkyl-chlorosilane is converted by water into the corresponding silanol. The silanol reacts selectively with fluoride to form fluorosilane, which can be extracted from the acidified solution with an organic solvent. The extracted fluoro-compound is determined quantitatively by means of G.C. as described by Fresen et al. (Pharma.Weekblad 103,909,1968)

Procedure

Preparation of Solution (A)

Weigh 0.4422 gm of NaF. Transfer to a 1000 ml volumetric flask and fill to volume with deionized water (0.001 gm Fluorine/5 ml).

Preparation of Solution (B)

Weigh about 0.15 gm of n-pentane. Transfer to a 250 ml volumetric flask and fill up to volume with toluene (0.003 gm/5 ml).

Preparation of solution (C)

Pipette 5 ml of fluoride solution (A) into a screw-cap tube, add 8 ml of conc. HCl and 5 ml of deionized water. Cap and mix. Add 2 ml of trimethylchlorosilane, cap and mix. Let stand for 15 minutes. Pipette in 5 ml of n-pentane solution (B), mix and inject 0.6 ul of top layer.

Determination of Total Soluble Available Fluorine in Dentifrice

Weigh accurately 10.0 gm \pm 1.0 gm of dentifrice into a centrifuge tube. Add approximately 20 - 30 ml of deionized water, slurry with a glass rod and centrifuge for 10 minutes.

Decant the supernatant liquid into a 100 ml volumetric flask. Repeat this extraction process an additional two times and bring the volume of the supernatant liquid up to 100 ml with deionized water.

Pipette 10 ml of the above solution into a screw-cap tube. Add 8 ml of conc. HCl and immerse in a boiling water bath for 1 minute and let stand for 10 minutes. Then cool under running water.

Add 1 ml trimethylchlorosilane, cap, mix and let stand for 15 minutes. Pipette 5 ml of n-pentane solution (B) into the test tube, shake and inject 0.6 ul of upper layer.

Calculation:

In the Standard Solution (C):

Factor =

$$\frac{\text{peak height of F}^- \text{ (TMFS)}}{\text{peak height of n-Pentane}} \times \frac{\text{gm of n-Pentane in 5 ml of Solution (B)}}{\text{gm of F}^- \text{ in 5 ml of Solution (A)}}$$

In the Dentifrice Sample:

gm of Fluorine =

$$\frac{\text{peak height of F}^- \text{ (TMFS)}}{\text{peak height of n-Pentane}} \times \frac{\text{gm of n-Pentane in 5 ml of Solution (B)}}{\text{Factor}}$$

$$\% \text{ Fluorine} = \frac{\text{gm of Fluorine} \times 10}{\text{weight of sample}} \times 100$$

$$\text{PPM of Fluorine} = \% \text{ Fluorine} \times 10,000$$

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STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #15

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE
IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #15DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE
IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICEObjective:

The objective of this method is to determine the total soluble available fluorine found in a sodium monofluorophosphate dentifrice.

| <u>Reagents</u> | <u>Suggested Type or Source</u> |
|-------------------------------|---------------------------------|
| Sodium Hydroxide 4N | Baker Analyzed Reagent |
| Polyethylene Centrifuge Tubes | S.G.A. |
| Deionized Water | |
| Volumetric Flasks | S.G.A. |
| Glass Beaker | S.G.A. |
| HCl Conc. | Baker Analyzed Reagent |
| Pipettes | S.G.A. |
| Sodium Acetate | M.C.B. |

| <u>Apparatus</u> | <u>Suggested Type or Source</u> |
|------------------------------|---------------------------------|
| Centrifuge Clinical | Damon/IEC Division |
| Ionalyzer Specific Ion Meter | Orion Research Inc. |

Procedure:

Weigh accurately $8-12 \text{ gm} \pm 0.2 \text{ gm}$ (w) of dentifrice into a glass beaker, slurry with 15-20 ml of 4 N sodium hydroxide and transfer carefully to a polyethylene centrifuge tube.

Centrifuge 5 minutes and decant into a polyethylene beaker. Slurry the residue again with 10-15 ml of deionized water. Centrifuge 5 minutes, decant and add to previous supernatant. Once more, slurry the residue with 10-15 ml of deionized water, centrifuge 5 minutes, decant and add to previous supernatant.

Acidify the combined supernatants with conc. HCl in small portions to a pH of 0.0 - 0.5. Add 2 ml excess of conc. HCl, cover and let stand 5-24 hours at room temperature. Transfer the solution quantitatively to a 100 ml volumetric flask and dilute to volume with deionized water.

Pipette 20 ml of the diluted sample solution into a 250 ml volumetric flask and again dilute to volume with deionized water.

Pipette 10 ml of this solution into a 100 ml volumetric flask, dilute to volume with sodium acetate solution (15% w/v) and mix thoroughly.

Standardize the specific ion meter by setting the center scale at 1 ppm fluorine and the upper end of the scale at 10 ppm fluorine using the two NaF solutions.

Using the meter, determine the reading in ppm (A) of the sample solution, immersing the specific electrode in about 40 ml of the solution contained in a polyethylene beaker and making due allowance for the electrode response time.

Calculation

$$\text{Total Soluble Available Fluorine (ppm)} = \frac{A \times 12,500}{W}$$

Where: A = ppm fluorine from meter reading
W = weight of dentifrice taken